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NOTE

Squamous cell carcinoma in a wild European bullhead *Cottus gobio*

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ABSTRACT: A pharyngeal tumor was found in a wild European bullhead. The tumor mass appeared underneath the operculum and was bilobed. The major neoplastic component showed diffuse epithelial squamous differentiation. Crossmonn's trichrome allowed identification of connective tissues whereas no neoplastic cells were stained. Periodic acid-Schiff was negative within the mass, and Giemsa did not show any further diagnostic significance. Immunohistochemistry showed diffuse positive cytoplasmic staining of the neoplastic population with an anti-human pancytokeratin antibody. Vimentin was negative and exclusively stained the stroma. On the basis of the morphological and immunohistochemical results, a squamous cell carcinoma was diagnosed.

KEY WORDS: Carcinoma · Pharynx · *Cottus gobio* · Immunohistochemistry

INTRODUCTION

In comparison with other countries, in Italy there are few historic records describing the occurrence of tumors in wild fish. Recently, sporadic reports have identified tumors in wild marine species (Marino et al. 2010b,c), whilst data on tumors in wild freshwater fishes are scarce or only available in the grey literature. Different etiologies, such as immunological, biochemical, nutritional factors and oncogenic viruses, have been suggested as tumor initiators. The frequency of neoplasms observed in fishes is often associated with virus and/or aquatic pollution (Couch & Harshbarger 1985). Environmental epizootics of skin neoplasia in brown bullhead with incidences of papilloma, carcinoma, and melanoma have been attributed to exposure to complex mixtures of chemical pollutants, particularly polycyclic aromatic hydrocarbons (Kim et al. 1989, Smith et al. 1989, Baumann et al. 1991, Bowser et al. 1991, Bunton 1996, Harshbarger & Clark 1990).

Furthermore, the presence of tumors in fish populations has been used as an indicator of environmental quality in saltwater and freshwater ecosystems (Smith et al. 1994, Grizzle & Goodwin 1998). Tumors of the mouth (Honma et al. 2003, Vijayakumar et al. 2014) and pharynx (Manera & Biavati 1999) have rarely been reported in fishes. They are generally tumors with odontogenic or osteogenic origin. Pharyngeal tumors were instead described in fish exposed to carcinogenic substances in experimental conditions (Chen et al. 1996). Epithelial tumors have been reported on lips (Marino et al. 2010a) but never in the oral cavity.

The European bullhead *Cottus gobio* Linnaeus, 1758, also known as freshwater sculpin and common bullhead, is a small demersal freshwater species belonging to the Cottidae family. It inhabits lakes, stony streams and the rivers of most of the European countries where the flow is moderate and the water is cool and oxygen-rich (Tomlinson & Perrow 2003). Few data are available in the literature on diseases of this ichthyic species. In this report, we describe an episode of neoplasia observed in a bullhead, a very sensitive fish to adverse habitat conditions, captured in a well-oxygenated stream with clear shallow water and a hard substrate of gravel and stones.

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MATERIALS AND METHODS

During a sampling to investigate the health status of some wild populations of white-clawed crayfish (*Austropotamobius pallipes* complex) carried out in Belluno Province of the Veneto region (northern Italy), 6 adult bullhead *Cottus gobio* were captured by electro-fishing in the Valturcana stream close to Puos d'Alpago (46° 8′ 21″ N, 12° 22′ 54″ E). The water parameters were as follows: temperature 16.8°C, pH 7.8, conductivity 685 µS cm^{-1}, dissolved oxygen 9.0 mg l^{-1} (98.7%). All fish were examined for externally visible pathological conditions. An irregular bilobed nodular mass was detected protruding underneath the left operculum of one subject, which was afterwards submitted to complete necropsy and histological examination. Tissue samples obtained from all organs as well as portions of the mass were fixed in 10% buffered formalin (for at least 24 h), trimmed into microcassettes and embedded in paraffin wax for histological examination. Sections of 5 µm were stained with haematoxylin and eosin, Crossmonn's trichrome, periodic acid-Schiff (PAS) and Giemsa. Immunohistochemistry was performed on neoplastic tissue sections. Specifically, anti-pancytokeratin (AE1/AE3), a general marker of epithelial cell cytoskeleton as well as of epithelial tumors, and anti-vimentin (V-9), a general marker for mesodermic cells and mesenchymal tumors, polyclonal antibodies (Dakocytomation) were used on an automated immunostainer (Ventana Benchmark XT, Roche). A standard immunohistochemical protocol was performed with heat-induced antigen retrieval (30 min at 95°C) and primary antibody (1:100 dilution) incubation at 37°C for 16–18 min. Positive controls were represented by internal epithelial (oral cavity) and mesenchymal (connective) tissues. Negative controls were obtained by replacing the primary antibody with the Ventana antibody diluent (Roche).

RESULTS

The protruding bilobed mass was ~2 cm in diameter and covered by intact skin. The neoplasm filled the gill chamber extending from underneath the operculum to the caudal margin of the cavity, and medially it extended into the dorsal potion of the oral cavity (Fig. 1). It was not encapsulated and not well demarcated and it infiltrated adjacent tissues.

A portion of the lesion was attached to the medial margin of the operculum while caudally 2 gill arches were incorporated into the mass. The mass was homogeneously white, compact, with a multinodular smooth surface. No further tumorous lesions were observed in other tissues or organs such as gills, gut, liver, kidney, spleen and heart, and no other significant lesions were detected. In the histological examination, the lesion had irregular not well-demarcated and infiltrative margins delineated by a stratified keratinizing epithelium that was continuous with the epithelium on the inner surface of the gill chamber (Fig. 2). The neoplastic tissue infiltrated the musculature below the operculum and the proximal margin of the pseudobranch. The multilobular mass was composed of irregular sheets and nests of ovoid to polygonal cells admixed with stromal tissue. The cells had poorly defined margins, a moderate to abundant slightly eosinophilic cytoplasm, and round to oval vesicular nuclei. Multifocally, the cells cytoplasm showed a squamous differentiation, and keratinised foci with occasional formation of keratin pearls-like structures were evident (Fig. 2B). Occasionally, the cells showed a palisading pattern

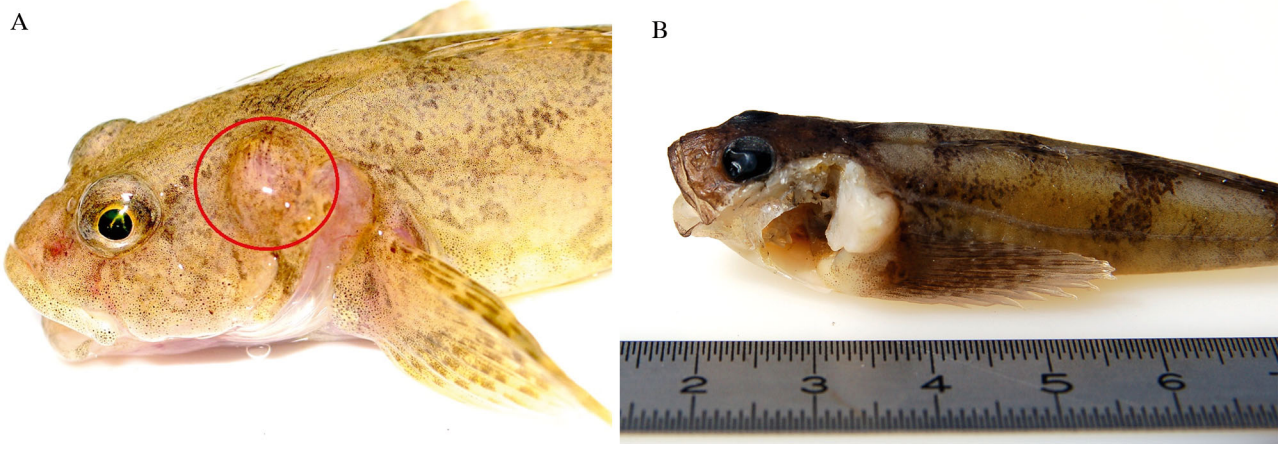

Fig. 1. *Cottus gobio*. (A) Bulging mass underneath the operculum (red circle). (B) Section of the neoplasm growing within the pharyngeal and gill cavity underneath the gill operculum

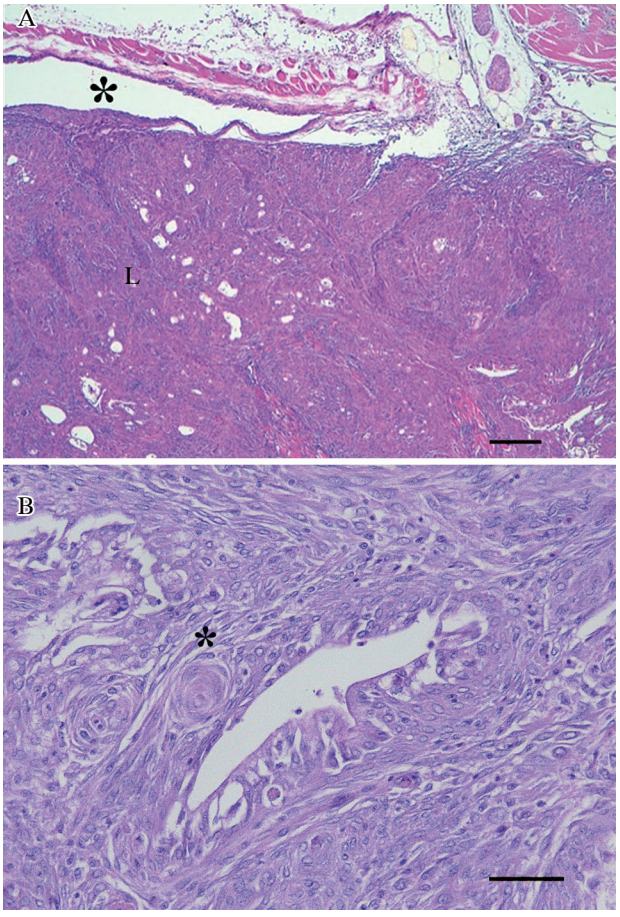

Fig. 2. *Cottus gobio*. (A) Neoplastic mass within the gill chamber. It is continuous with the gill chamber (*) epithelium and shows a slightly lobulated pattern and empty lacunae spaces (L) (H&E). (B) Higher magnification of the mass showing moderate anisocytosis and anisokariosis, as well as a lacuna surrounded by palisade cells with squamoid differentiation and keratin pearl-like structures (*) (H&E). Scale bars = 100 µm

which appeared to lie on the basal lamina as shown by PAS. They showed marked spongiosis and that the cells had apically located nuclei. Disseminated lacunae were also evident, occasionally surrounded by palisading cells (Fig. 2). Anisokaryosis and anisocytosis were moderate, and mitoses varied from 1 to 2 per high power field. Multifocally, the neoplastic tissue exhibited cells with pyknotic nuclei and necrotic areas. Severe hyperaemia and a diffuse moderately mixed inflammatory infiltrates (lymphocytes, plasmacells and macrophages) were distributed within the neoplastic stroma characterized by hypertrophic, hyperplastic, and irregularly oriented fibroblasts (scirrous reaction). The superficial epithelium of the gill chamber showed diffuse exocytosis of mononuclear inflammatory cells and multifocal ulceration.

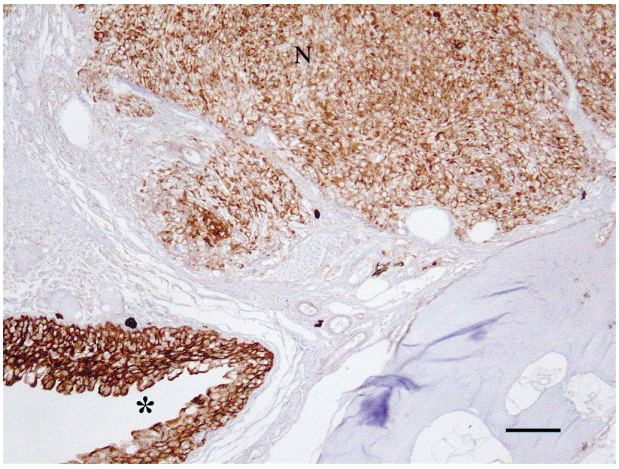

Fig. 3. *Cottus gobio*. Neoplastic mass within the gill chamber. Diffuse positivity of the neoplastic cells (N) for anti-human pancytokeratin antibody. The gill chamber epithelium (*) was diffusely positive and used as an internal control (IHC). Scale bar = 160 µm

By histochemistry, the Crossmonn's trichrome allowed identification of connective tissues; no neoplastic cells were stained. PAS reaction was negative within the mass, and Giemsa stain did not show any further diagnostic features. Immunohistochemistry showed diffuse positive cytoplasmic staining of the neoplastic population with an anti-human pancytokeratin antibody (Fig. 3). Vimentin was negative and exclusively stained the stroma. On the basis of the morphological and immunohistochemical results, a squamous cell carcinoma (SCC) could be diagnosed. No metastases were observed.

DISCUSSION AND CONCLUSION

Gross, histological, histochemical and immunohistochemical findings were consistent with a diagnosis of a malignant epithelial neoplasm. An odontogenic tumor (i.e. ameloblastoma) was considered in the early differential diagnosis, although a carcinoma of other origin (glandular or surface epithelium) could not be ruled out. The major neoplastic component showed squamous differentiation and pancytokeratin-positive staining. For this reason, the origin of the neoplasm was therefore considered epithelial. Since other typical aspects of odontogenic tumors (regularly distributed stellate mesenchymal cells or stellate reticulum) were not evident, the early diagnosis of an odontogenic neoplasm was not confirmed. Association with the overlaying skin was not detected, and no tubular pattern was clearly identifiable excluding a glandular origin.

A final diagnosis of SCC arising from the gill chamber epithelium was made. Anaplasia and aggressive behavior were less evident than in typical SCC diagnosed in other species (Karingalakkandy Poochirian et al. 2011); however, the described features of pancytokeratin positivity, keratin-pearl formation, and infiltrative activity were indicative of a carcinoma with squamous differentiation. Many of the neoplasms observed in fish have a mesenchymal origin (Harshbarger & Slatick 2001). Particularly, the gill operculum is often affected by branchioblastomas (Chen et al. 1996, Knüsel et al. 2007) and osteochondromas arising from gill arches (Nash & Porter 1985, Manera & Biavati 1999). In the case described here, neither bone nor cartilage were evident within the lesion.

Papillomas described in brown bullheads in New York State, USA (Poulet et al. 1994) were more commonly multiple (89%) than solitary (11%), as was observed in the present study, and showed a typical hyperplastic exophytic growth that was not evident in the tumor described here. Considering that only few cases of SCC in wild fish have been described (e.g. Hanjavanit & Mulcahy 2004), the present case will facilitate development of a general description of the pathological characteristics of this tumor-type in wild freshwater fish. Further extensive epidemiological/monitoring studies conducted at sites in different locations and performed on appropriate sample numbers are in progress to evaluate the presence of chemical residues and to propose fish that can serve as biological indicators of the presence of potential carcinogens in the water environment. Moreover, as already suggested by Marino et al. (2016), this paper confirms that antibodies produced for identification of human tissues can be a useful tool in fish oncology to determine the cell origin of tumors.

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